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O papel de genes envolvidos em vias de neuroinflamação no TDAH e fenótipos relacionados

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

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RESUMO

O Transtorno de Déficit de Atenção e Hiperatividade (TDAH) é um transtorno do neurodesenvolvimento caracterizado por sintomas de desatenção, impulsividade e hiperatividade que possui etiologia multifatorial. As bases biológicas do TDAH incluem desenvolvimento neural alterado, desregulação da neurotransmissão, aumento do estresse oxidativo e ativação glial, alterações na neurogênese e sinaptogênese. Nesse sentido, a neuroinflamação tem sido proposta como um mecanismo biológico potencialmente envolvido em alterações no funcionamento encefálico que podem estar associadas a susceptibilidade a transtornos psiquiátricos. Processos de resposta neuroinflamatória já foram associados anteriormente com diversos transtornos psiquiátricos e doenças neurodegenerativas. Assim, o presente trabalho tem como objetivo avaliar o papel de vias de genes relacionados à neuroinflamação no TDAH, suas principais comorbidades e no desempenho cognitivo. A amostra é composta por 407 adultos diagnosticados com TDAH, de acordo com o DSM-5, oriundos da divisão de adultos do Programa de Deficit de Atenção e Hiperatividade (ProDAH) e 463 controles adultos, oriundos de Banco de Sangue do Hospital de Clínicas de Porto Alegre. A genotipagem das amostras foi realizada utilizando o Infinium PsychArray-24 BeadChip. O controle de qualidade (QC), análise de componentes principais (PC) e procedimentos de imputação de genótipos foram implementados usando o pipeline Ricopili seguindo parâmetros padrão. Foram selecionados 23 gene-sets de vias relacionadas à neuroinflamação a partir de uma busca realizada no *Molecular Signature Database* (MsigDB). As análises de gene-sets foram executadas no software MAGMA. Sexo, idade e os primeiros 5 PCs foram incluídos como covariáveis. A correção de múltiplos testes foi aplicada por FDR, considerando todos os conjuntos de genes e desfechos testados. Para interpretar as associações encontradas em termos de confiabilidade, foram gerados gráficos QQ-plot, em que os escores Z de resíduos dos genes em cada conjunto são plotados em relação aos seus valores esperados. Associações nominais foram observadas para várias vias neuroinflamatórias, especialmente aquelas relacionadas a moléculas de orientação axônica com diagnóstico de TDAH e quociente de inteligência. As que se mostraram mais robustas, entretanto, foram aquelas envolvendo o Transtorno de Ansiedade Generalizada e o Transtorno por Uso de Substâncias, nas quais uma inspeção mais aprofundada indicou que as vias estavam em sua totalidade associadas a esses transtornos, apresentado sinais mais fortes. Em geral, nossos achados sugerem que a neuroinflamação pode ser um possível mecanismo fisiopatológico comum dos principais transtornos psiquiátricos e características associadas. Para trabalhos futuros, pretendemos realizar essas análises de gene-sets em uma amostra populacional, para avaliar como se comportam os resultados quando o transtorno avaliado não está em comorbidade com TDAH. Também pretendemos utilizar análises de escores de risco poligênico (PRS, e PRS-set) como uma abordagem alternativa para investigar a associação de genes relacionados à neuroinflamação e TDAH, comorbidades e desempenho cognitivo.

Palavras-chave: neuroinflamação; TDAH; comorbidades; moléculas de orientação axonal

ABSTRACT

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by symptoms of inattention, impulsivity and hyperactivity that has a multifactorial etiology. The biological underpinnings of ADHD include altered neural development, dysregulation of neurotransmission, increased oxidative stress and glial activation, changes in neurogenesis and synaptogenesis. In this sense, neuroinflammation has been proposed as a biological mechanism potentially involved in changes in brain functioning that may be associated with susceptibility to psychiatric disorders. Neuroinflammatory response processes have previously been associated with several psychiatric disorders and neurodegenerative diseases. Thus, the present study aims to evaluate the role of neuroinflammation-related gene pathways in ADHD, its main comorbidities and cognitive performance. The sample consists of 407 adults diagnosed with ADHD, according to the DSM-5, from the adult division of the Attention Deficit Hyperactivity Disorder Program (ProDAH) and 463 adult controls from the Hospital de Clínicas de Porto Alegre's blood center. Genotyping of samples was performed using the Infinium PsychArray-24 BeadChip. Quality control (QC), principal component analysis (PC) and genotype imputation procedures were implemented using the Ricopili pipeline following standard parameters. Twenty-three gene-sets of pathways related to neuroinflamation were selected from a search performed in the Molecular Signature Database (MsigDB). Gene-set analyzes were performed using the MAGMA software. Sex, age and the first 5 PCs were included as covariates. FDR multiple test correction was applied considering all gene sets and outcomes tested. To interpret the associations found in terms of reliability, QQ-plot plots were generated, in which the Z-scores of gene residues in each set are plotted against their expected values. Nominal associations were observed for several neuroinflammatory pathways, especially those related to axonal guidance molecules with ADHD diagnosis and intelligence quotient. The ones that were more robust, however, were those involving Generalized Anxiety Disorder and Substance Use Disorder, in which a closer inspection indicated that the pathways were in their totality associated with these disorders, presenting the strongest signals. Overall, our findings suggest neuroinflammation as a possible common pathophysiological mechanism of major psychiatric disorders and associated features. For future work, we intend to carry out these gene-set analyzes in a population sample, to assess how the results behave when the assessed disorder is not comorbid with ADHD. We also intend to use polygenic risk score (PRS and PRS-set) analyzes as an alternative approach to investigate the association of genes related to neuroinflammation and ADHD, comorbidities, and cognitive performance.

Keywords: neuroinflammation; ADHD; comorbidities; axonal guidance molecules

SUMÁRIO

1 INTRODUÇÃO COMPREENSIVA

1.1 ASPECTOS GERAIS DO TDAH

O Transtorno de Déficit de Atenção e Hiperatividade (TDAH) é um transtorno psiquiátrico do neurodesenvolvimento caracterizado por sintomas de hiperatividade, desatenção e impulsividade. Sua prevalência é de 5% em crianças (POLANCZYK *et al.*, 2007) e 2,5% em adultos (FAYYAD *et al.*, 2017) e está comumente em comorbidade com outros transtornos psiquiátricos, como os transtornos opositivo desafiante, ansiedade generalizada, depressivo maior e por uso de substância. Além disso, o TDAH vem sendo clinicamente relacionado com doenças somáticas que apresentam comprometimentos imunológicos e inflamatórios (CHANG *et al.*, 2021).

O diagnóstico do TDAH requer avaliação de sintomas atuais e da infância (presentes antes dos 12 anos de idade) e é baseado no Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-5). Para que uma criança seja diagnosticada com TDAH, é necessário a manifestação de 6 ou mais sintomas que causem prejuízo em ao menos dois contextos de vida (ex: trabalho e responsabilidades diárias), enquanto que, para adultos, é necessária a presença de no mínimo 5 sintomas, como por exemplo, dificuldade de organização e dificuldade em manter atenção em tarefas prolongadas. Este transtorno possui 3 apresentações, classificadas de acordo com os sintomas presentes: desatenta, hiperativa-impulsiva ou combinado (BÉLANGER *et al.*, 2018).

Indivíduos com TDAH apresentam uma ampla gama de prejuízos em esferas pessoais, sociais e profissionais, como fracasso escolar, lesões causadas por acidentes e comportamentos autolesivos (LEFFA; TORRES; ROHDE, 2018). A neurobiologia do TDAH é complexa e parece ser consequência de vários fatores neurofisiológicos disfuncionais, como os sistemas de neurotransmissão monoaminérgicos, principalmente os dopaminérgicos e noradrenérgicos (ARNSTEN; PLISZKA, 2011). As bases biológicas do TDAH incluem desenvolvimento neural alterado, desregulação da neurotransmissão, aumento do estresse oxidativo e ativação glial, alterações na neurogênese e sinaptogênese. Além disso, estudos de neuroimagem sugerem diferenças em áreas cerebrais que regulam o funcionamento executivo, processamento de recompensas e informações (FARAONE; LARSSON, 2019).

1.2 ETIOLOGIA DO TDAH

1.2.1. Fatores ambientais

A etiologia do TDAH é multifatorial, ou seja, tanto fatores ambientais quanto genéticos contribuem para a manifestação dos sintomas. Fatores pré-natais (como tabagismo e alcoolismo materno durante a gravidez e obesidade materna), perinatais (como prematuridade e baixo peso ao nascer), bem como o contexto social na infância, incluindo adversidades sofridas, estão entre os principais fatores ambientais associados com ao TDAH (POSNER; POLANCZYK; SONUGA-BARKE, 2020).

Uma revisão publicada no ano de 2021 reuniu estudos que demonstram que a exposição a um ambiente materno imunologicamente desregulado pode afetar o neurodesenvolvimento fetal. Algumas evidências epidemiológicas que suportam essa hipótese demonstram que infecções virais, bacterianas e protozoárias aumentam o risco de distúrbios neurológicos e neuropsiquiátricos; infecções geniturinárias maternas aumentam as chances de prole com TDAH em 26-36% e gestantes com infecções respiratórias virais apresentam risco três vezes maior de prole com TDAH. Além disso, doenças autoimunes e asma materna foram associadas com transtorno do espectro autista e TDAH (HAN *et al.*, 2021).

1.2.2. Fatores genéticos

Estima-se que cerca de 80% da variabilidade observada na população seja decorrente de variação genética, conceito denominado herdabilidade. Assim, faz-se necessária a investigação de fatores genéticos associados à susceptibilidade ao TDAH e seus desfechos clínicos. Nesse sentido, o estudo de associação por varredura genômica (GWAS, do inglês *Genome-Wide Association Study*) mais recente publicado para o TDAH revelou 27 loci significativamente associados com o transtorno em uma amostra de 38.691 casos e 186.843 controles. As variantes de risco associadas com o TDAH estão implicadas em prejuízo na cognição, incluindo raciocínio verbal, e em uma série de funções executivas. A maioria delas está localizada em genes expressos no córtex frontal, córtex pré-frontal e mesencéfalo (como por exemplo o *FOXP1* e *FOXP2*), justificando déficits nas áreas motoras, no processamento de recompensas e nas funções executivas e cognitivas (DEMONTIS *et al.*, 2022). É interessante destacar que as associações encontradas não apontam para genes candidatos anteriormente implicados no TDAH, como é o caso do *BAIAP2* associado a etiologia do transtorno em um estudo de meta-análise (BONVICINI et al., 2016). No entanto, a herdabilidade molecular

(proporção da variabilidade fenotípica explicada por variantes comuns avaliadas em GWAS) encontrada nesse estudo foi de 14%, com estimativa de que 7000 variantes expliquem em torno de 90% dessa herdabilidade molecular, um número muito maior do que aquelas significativamente associadas. Isso significa que um número considerável de variantes genéticas ainda não foram identificadas pelos GWAS de TDAH e podem se tornar significativas à medida que o tamanho amostral aumenta.

Estudos genômicos também fornecem evidências de que o TDAH apresenta arquitetura genética compartilhada com uma ampla gama de fenótipos, incluindo domínios psiquiátricos, cognitivos, comportamentais e metabólicos, o que ajuda a entender a associação clínica observada do TDAH com esses fenótipos. Existe uma correlação genética negativa entre o TDAH e diferentes domínios neurocognitivos, incluindo atenção e memória de trabalho, e também desempenho acadêmico, o que corrobora a relação entre TDAH e prejuízo no funcionamento cognitivo e executivo (ADHD WORKING GROUP OF THE PSYCHIATRIC GENOMICS CONSORTIUM (PGC) *et al.*, 2019; BRAINSTORM CONSORTIUM *et al.*, 2018; DEMONTIS *et al.*, 2022). Por outro lado, o TDAH apresenta correlações genéticas positivas com outros transtornos psiquiátricos (por exemplo, transtorno depressivo maior, transtorno do espectro autista, transtorno bipolar e esquizofrenia) e com outros fenótipos associados, como neuroticismo, sintomas de depressão, insônia, tabagismo e uso de cannabis (ADHD WORKING GROUP OF THE PSYCHIATRIC GENOMICS CONSORTIUM (PGC) *et al.*, 2019; ARTIGAS *et al.*, 2020; BRAINSTORM CONSORTIUM *et al.*, 2018; DEMONTIS *et al.*, 2022). No caso de doenças metabólicas e variáveis relacionadas, a genética do TDAH correlacionou-se positivamente com a genética dos parâmetros antropométricos, sobrepeso/obesidade, diabetes tipo 2 e triglicerídeos, enquanto negativamente correlacionada com a genética do HDL (ADHD WORKING GROUP OF THE PSYCHIATRIC GENOMICS CONSORTIUM (PGC) *et al.*, 2019; DEMONTIS *et al.*, 2022). O TDAH também apresenta uma correlação positiva com diversas doenças e fenótipos relacionados ao sistema imunológico tais como asma, artrite reumatoide e proteína C-reativa (TYLEE *et al.*, 2018).

1.3 NEUROINFLAMAÇÃO

A neuroinflamação, processo inflamatório que ocorre no tecido neural, é uma das principais linhas de defesa do sistema nervoso central (SNC) contra patógenos, protegendo e restaurando o mesmo em casos de infecções e lesões. Fatores como insulto inicial (qualquer

patógeno que desencadeia a resposta inflamatória), predisposição genética, idade e memória imunológica podem desencadear a ativação das vias neuroinflamatórias (SHABAB *et al.*, 2017).

As microglias são as células de defesa do sistema nervoso central, análogas aos macrofágos, que podem ser ativadas na presença de patógenos, por danos nos tecidos, estresse oxidativo ou pela presença de neurotoxinas. Uma vez ativadas elas são capazes de liberar citocinas, radicais livres de oxigênio e aumentar sua atividade fagocitária. Em casos de neuroinflamação crônica, estas células são capazes de permanecer ativadas por longos períodos causando neurodegeneração (SHABAB *et al.*, 2017). A ativação microglial não tem seu papel somente na produção de citocinas, mas também no desenvolvimento inicial do cérebro e do SNC, bem como em sua conectividade e plasticidade (SALTER; BEGGS, 2014).

Células gliais produzem moléculas de orientação de axônios como netrinas, semaforinas e efrinas, mesmo após a conclusão do desenvolvimento do SNC. Estas moléculas, já conhecidas pelo seu papel no neurodesenvolvimento, recentemente foram relacionadas a respostas do sistema imune no SNC pós natal, uma vez que sua expressão pode ser alterada por estímulos inflamatórios, tendo um papel protetor ou prejudicial no processo de neuroinflamação (LEE *et al.*, 2019). Além disso, durante o desenvolvimento, as células gliais são altamente sensíveis ao ambiente inflamatório, pois alterações nos marcadores de inflamação alteram os gradientes quimioatrativos e o funcionamento destas células de suporte (DUNN; NIGG; SULLIVAN, 2019).

Há evidências de que existe uma comunicação entre as células imunológicas e as células do SNC, capaz de modular a plasticidade sináptica e a neuroimunidade (ALMEIDA *et al.*, 2020). Além disso, a neuroinflamação contribui para o recrutamento de células inflamatórias periféricas e aumenta a permeabilidade da barreira hematoencefálica (KEMPURAJ *et al.*, 2017).

1.4 RELAÇÃO DO TDAH COM MECANISMOS INFLAMATÓRIOS E NEUROINFLAMATÓRIOS

A inflamação e seus mecanismos patológicos estão relacionados com a fisiopatologia de doenças psiquiátricas através de mecanismos como ativação glial, aumento do estresse oxidativo, redução do suporte neurotrófico e alteração do metabolismo de neurotransmissores (LEFFA; TORRES; ROHDE, 2018).

As evidências do envolvimento de mecanismos relacionados ao sistema imune e processos inflamatórios na fisiopatologia do TDAH são provenientes principalmente de estudos epidemiológicos que relatam uma alta comorbidade entre TDAH e distúrbios do sistema imune, como por exemplo, asma, rinite, dermatite atópica, conjuntivite e psoríase (CHANG *et al.*, 2021). Além disso, estudos avaliando marcadores inflamatórios mostram que indivíduos com TDAH apresentam níveis aumentados de anticorpos contra células de Purkinje, anticorpos anti-gânglios basais e anticorpos contra transportadores de dopamina, além de níveis séricos aumentados de IL-6 e IL-10 (LEFFA; TORRES; ROHDE, 2018).

Ainda que genes envolvidos em vias de inflamação não tenham sido identificados no GWAS de TDAH, o que talvez possa ser explicado pela falta de poder estatístico e que muitas associações podem surgir com o aumento do tamanho amostral, a importância desses genes já foi evidenciada por estudos de gene candidato. Estudos que buscaram mostrar associação de polimorfismos de genes de citocinas com o TDAH relataram aumento da transmissão do alelo *IL-1Ra* de 4 repetições e diminuição da transmissão do alelo de 2 repetições em crianças com TDAH. O alelo *IL-1Ra* de 4 repetições foi associado a um risco significativamente aumentado de TDAH (SEGMAN *et al.*, 2002). Um segundo estudo mostrou associação entre polimorfismos nos genes *IL-6* e *Fator de Necrose Tumoral Alfa*, descrevendo uma diferença estatisticamente significativa na frequência alélica e genotípica destes polimorfismos entre grupos de pessoas diagnosticadas com TDAH e controles (DRTILKOVA *et al.*, 2008).

Considerando as evidências que apoiam uma possível relação biológica entre a neuroinflamação e o TDAH (comorbidade com distúrbios inflamatórios, diferenças nos níveis de biomarcadores inflamatórios e estudos genéticos), bem como a influência do contexto ambiental em relação à resposta inflamatória durante o neurodesenvolvimento, buscamos avaliar a relação entre genes envolvidos nas vias de processos neuroinflamatórios e o TDAH e desfechos clínicos relacionados.

1.3 OBJETIVOS

1.3.1 OBJETIVO GERAL

Avaliar a associação de genes envolvidos em vias biológicas do sistema imune e processos neuroinflamatórios com o TDAH, comorbidades e características associadas através de uma análise de gene-sets.

1.3.2 OBJETIVOS ESPECÍFICOS

a) Selecionar vias biológicas (ou *gene-sets*) envolvidas no processo de neuroinflamação e moléculas relacionadas a partir do *Molecular Signatures Database*;
b) Testar a associação dos *gene-sets* selecionados com a susceptibilidade ao TDAH;
c) Avaliar a associação dos *gene-sets* selecionados com fenótipos relacionados ao TDAH, incluindo comorbidades e desempenho cognitivo.

2 ARTIGO CIENTÍFICO

Em preparação para submissão na revista *Brazilian Journal of Psychiatry* (IF = 6.328)

Neuroinflammation-related gene pathways in Attention-Deficit/ Hyperactivity Disorder and associated phenotypes

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ABSTRACT

Objective: A possible link between the immune system and inflammatory response and the pathophysiology of major psychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD), has been suggested. This study aims to identify biological molecular pathways related to neuroinflammatory response and test their association with ADHD and common traits associated with ADHD. **Methods:** The sample is composed of 407 adult patients with ADHD and 463 controls. Genotyping of the samples was performed using the Infinium PsychArray-24 BeadChip. The Molecular Signatures Database was used to search for gene-sets related to neuroinflammation. Gene-set analyses were performed to test the association of the selected pathways with ADHD diagnosis, its major comorbidities, and cognitive performance in the ADHD sample using the MAGMA software. **Results:** Nominal associations were observed for several neuroinflammatory-related pathways with ADHD diagnosis, comorbidities, and intelligence quotient. **Conclusions:** Further inspection of the associations suggests that the stronger signals were those involving generalized anxiety and substance use disorder, especially involving pathways related to axon guidance molecules. In general, our findings suggest neuroinflammation as a possible common pathophysiological mechanism of major psychiatric disorders and associated impaired traits.

Keywords: neuroinflammation; ADHD; comorbidities; axonal guidance molecules

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by symptoms of inattention, hyperactivity, and impulsivity, with a prevalence of 2.5% in adults[1] and 5% in children[2]. The etiology of the disorder is multifactorial, that is, both environmental and genetic factors contribute to the manifestation of symptoms. Biological underpinnings implicated in ADHD include altered neural development, dysregulation of neurotransmission, increased oxidative stress and glial activation, impaired neurogenesis and synaptogenesis[3]. In this sense, neuroinflammation has been proposed as a mechanism that, by leading to alterations in microglia, astrocytes, cytokines, and other immune-related components, can influence neurodevelopmental processes, including synaptic plasticity and neurogenesis[4].

Several lines of evidence link ADHD to the immune system and inflammatory response. Differences in peripheral inflammatory markers levels have been reported in patients with ADHD, suggesting an increased inflammation in these individuals compared to controls. Among the main markers implicated are interleukins, C-reactive protein, interferon-gamma, and tumor necrosis factor (TNF)-alpha[5]. Besides, ADHD subjects also present increased antibodies against Purkinje cells, anti-basal ganglia antibodies, and antibodies against dopamine transporters[6]. Clinically, ADHD is associated with inflammatory conditions and autoimmune diseases, including asthma, rhinitis, atopic dermatitis, psoriasis, and conjunctivitis[7]. This clinical association is supported by genetic studies showing a high genetic correlation between ADHD and immune-related conditions such as asthma and psoriasis[8]. Also, specific genes related to inflammatory pathways have been associated with ADHD, such as polymorphisms in *IL-1*[9] and *IL-6* genes[10], *TNF-alpha*.[10]

It is noteworthy that major psychiatric disorders and related traits present a considerable proportion of shared genetic background and inflammation has emerged as an important component in this context, being one of the most robust evidence, the associations reported in the latest schizophrenia GWAS.[11] A relationship between inflammation and depression, anxiety, intelligence, and other psychiatric-related traits have also been reported[4,12]. In this sense, neuroinflammation might represent a common pathophysiological mechanism underlying these phenotypes.

Although the evidence supports a link between (neuro)inflammatory processes and ADHD, and other psychiatric traits, the molecular pathways underlying such association are poorly understood, lacking replicable genetic findings. While increasing efforts to obtain larger sample sizes to enhance the power of GWAS to reveal additional associations for ADHD are being made, the investigation of relevant pathways that might represent a common genetic architecture between clinically relevant comorbidities of ADHD can help to disentangle mechanisms underlying such associations. This study aims to identify biological pathways related to neuroinflammatory genes that comprise the genetic architecture of ADHD, and to test whether this genetic component is shared with common traits associated with ADHD.

METHODS

Subjects and diagnostic procedures

The sample of patients with ADHD is composed of 407 adults of both sexes who were diagnosed at the adult division of the ADHD outpatient program (ProDAHS-A) of Hospital de Clínicas de Porto Alegre (HCPA) according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). All participants were evaluated through an extensive

clinical protocol. The Portuguese version of the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS-E)[20], adapted for adults[13]for ADHD and oppositional defiant disorder (ODD) diagnoses. Symptom severity was evaluated through the Swanson, Nolan and Pelham scale IV (SNAP-IV). Comorbidities were assessed using the SCID-I (Structured Clinical Interview for DSM-IV) and the MINI (for Conduct and Antisocial Personality disorders). IQ evaluation was performed using the Weschler Adult Intelligence Scale (WAIS-R), using the vocabulary and block design subtests. All subjects are white Brazilians of predominantly European descent aged 18 years or older. The exclusion criteria included evidence of a clinically significant neurological disease that might affect cognition (such as a history of head trauma and/or epilepsy or dementia) and an estimated IQ below 70. More details on diagnostic procedures and patient characterization can be found in Grevet et al. 2005[21].

The control sample is composed of 463 adult healthy volunteers, of both sexes, from the blood donation center of HCPA with a negative screening for ADHD according to the 6-item Adult ADHD Self-Rated Scale Screener (ASRS)[14].

The sample is ascertained from a research project approved by the institutional review board of HCPA (IRB 0000921). All participants signed an informed consent form. This work was carried out following the Declaration of Helsinki.

Genotyping

Genotyping of the samples was performed using the Infinium PsychArray-24 BeadChip (Illumina, San Diego, CA, USA). Pre-imputation quality control (QC), principal components (PC) analysis and genotype imputation procedures were implemented using the Ricopili pipeline following default parameters (//sites.google.com/a/broadinstitute.org/ricopili/home). The European population of the 1000 Genomes Project Phase 1 was used as the reference panel

mapped to the GRCh37 build. Post-imputation QC was performed using the following settings for inclusion of variants or individuals: info score > 0.8, minor allele frequency > 10%, SNP and individual call rate > 95%, and Hardy-Weinberg equilibrium test with p-value > 1e-06.

Selection of gene-sets

The search for gene-sets related to neuroinflammation and related molecules was performed using *Molecular Signatures Database* (MsigDB). First, the keywords searched corresponded to general pathways of neuroinflammation. After that, a literature search was performed to include pathways related to important specific immune-related molecules that were not widely represented in the general neuroinflammation gene-sets[15]. The searched keywords were: "neuroinflammation", "neuroinflammatory", “cytokine”, “semaphorin”, “ephrin”, “netrin”, “TNF”, “tumor_necrosis_factor”. We selected gene-sets containing up to 200 genes. If the search retrieved a large number of gene-sets for the same keyword with a high proportion of overlapping genes between them, we selected those that better represent the pathway of interest based on the literature review and the number of genes. This strategy was adopted to refine the selection and include pathways that are more specific for the neuroinflammatory response and signaling.

The genes-sets included were: HP Neuroinflammation (17 genes); WP Neuroinflammation (11 genes); WP Neuroinflammation And Glutamatergic Signaling (136 genes); GOPB Neuroinflammatory Response (66 genes); Biocarta Cytokine Pathway (19 genes); GOBP Cytokine Production Involved In Immune Response (116 genes); GOPB Cytokine Production Involved In Inflammatory Response (55 genes); WP Cytokine And Inflammatory Response (25 genes); GOBP Semaphorin Plexin Signaling Pathway (41); GOBP Semaphorin Plexin Signaling Pathway Involved In Neuron Projection Guidance (14 genes);

REATCOME Sema4D In Semaphorin Signaling (24 genes); REATCOME EPH Ephrin Mediated Repulsion of Cells (50 genes); REATCOME EPH Ephrin Signaling (90 genes); REATCOME Ephrin Signaling (17 genes); GOBP Netrin Activated Signaling Pathway (10 genes); PID Netrin Pathway (32 genes); REATCOME Netrin 1 Signaling (49 genes); REATCOME Netrin Mediated Repulsion Signals (8 genes); WP NETRINUNC5B Signaling Pathway (50 genes); REATCOME TNF signaling (41 genes); WP TNFALPHA Signaling Pathway (89 genes); GOBP Tumor Necrosis Factor Mediated Signaling Pathway (99 genes); ST Tumor Necrosis Factor Pathway (28 genes).

Data analysis

The strategy used for the analysis of genomic data was the gene-set approach, in which variants of genes are grouped according to their participation in common biological pathways and analyzed together, in order to assess the effect of the pathway as a whole on the outcomes tested. Besides the case-control status, we also evaluated the effects of the gene-sets on common comorbidities and IQ within the ADHD sample. The first step of the gene-set approach is the SNP annotation to genes, for which we used gene locations for build 37 (hg19), setting a 35kb upstream and 10kb downstream window to include regulatory regions around genes. The second step is the gene-based analysis, which was performed using the principal components regression model, the default for raw genotype data. Finally, the gene-set analysis was performed using the competitive model, which tests if the association of the genes comprising a specific gene-set with the outcome of interest is stronger than other genes not included in the gene-set. Sex, age and the first 5 PCs were included as covariates. These analyses were performed using the MAGMA software[16]. FDR multiple testing correction was applied considering all the gene-sets and outcomes tested.

Set specific QQ-plots

QQ-plots for genes in each gene-set associated with an outcome were generated according to the procedures described by de Leeuw et al. (2018)[17], using MAGMA outputs and RStudio software v.3.4.0 (http://www.rstudio.com/). In the QQ-plots, the residual Z-scores of the genes in each set are plotted against their expected values. The expected values are based on all genes in the data, using the sample quantiles of the residual Z-scores. In these plots, an early and upwards deviation from the diagonal would indicate that the gene-set in its entirety contributes to the association. The confidence band is the likely range of chance deviation per individual gene, and a low proportion of genes exceeding this band is usually seen when there are many non-contributing genes.

RESULTS

The mean age of the sample of cases is 33.6 years and it is composed of 191 female participants, which comprises 46.9% of the total sample. The mean age of the control sample is 29.4 years and it is composed of 241 women, which comprises 52.1% of the total sample. Other characteristics of the sample, including the comorbidity profile and scores of IQ can be found in **Table 1**.

Competitive gene-set analyses

Of the 23 gene-sets analyzed, 5 showed a nominal association with the diagnosis of ADHD. When we analyzed the same gene-sets with ADHD-associated phenotypes, 2 had a

nominal association with generalized anxiety disorder (GAD), 2 with major depressive disorder (MDD), 2 with substance use disorder (SUD), 1 with oppositional defiant disorder (ODD), 5 with vocabulary subtest of IQ, and 2 with block design subtest of IQ (**Table 2**). None of the results survived the FDR correction.

Set specific (QQ-plots)

According to the recommendations described by de Leeuw et al. (2018)[17], QQ-plots for the associated gene-sets were generated to check for signs of confounding and outlier problems (see **Supplementary Figures**). Among the inspected QQ-plots, those showing nominal association between the Reactome EPH Ephrin Signaling gene-set (90 genes) and the WP Neuroinflammation and Glutamatergic Signaling gene-set (136 genes) and GAD presented the highest confidence that those pathways are associated in their entirety with the outcome (**Figure 1 - A** and **B**, respectively). **Figure 1-A** shows that there is an early deviation of the plotted residualised Z-scores from the diagonal line (dotted line) and 70% of the genes are above the confidence band (dashed line), suggesting strong associations (reflected by higher Z-scores) for most genes within the gene-set compared to what is expected by random distribution. **Figure 1-B** for QQ-plot of the WP Neuroinflammation and Glutamatergic Signaling association with GAD shows that although there are some genes below the diagonal line, they begin to deviate from both the diagonal line and the confidence band before the first percentile (represented by the black dots, dividing the genes within the set at 25%, 50% and 75%), and 80.1% of the genes exceeds the confidence band. This pattern also indicates a strong deviation and that the association is likely driven by a high proportion of genes within the set. A similar pattern can be seen for the association between SUD and the GOBP cytokine production involved in immune response gene-set. As shown in **Figure 1-C**, 78.4% of the genes exceed the confidence

band, indicating an association between the pathway in its entirety and the respective outcome. The remaining QQ-plots indicate confounding or that many non-contributing genes are present in the gene-set.

DISCUSSION

In an attempt to better understand the role of neuroinflammation in ADHD and related phenotypes, we used the gene-set approach to analyze different outcomes in a sample of adults with ADHD. Our findings provide suggestive evidence of a role of neuroinflammatory-related pathways on ADHD susceptibility, especially those related to axon guidance molecules. Such association was extended to common genetically correlated comorbidities of ADHD and cognitive performance, suggesting neuroinflammation as a possible common pathophysiological mechanism of major psychiatric disorders and associated impaired traits.

Although our results are of nominal significance, the inspection of the QQ-plots allowed us to further interpret the main findings and identify those representing the strongest signals of association. Among them, we can highlight the association of GAD in patients with ADHD with the ephrin signaling and the neuroinflammation and glutamatergic signaling pathways. These molecular pathways show a pattern of QQ-plot that indicates the pathways in their entirety are associated with the outcome and that the association is not being carried by only a few genes within the gene-set. These findings corroborate previous studies suggesting that neuroinflammation can cause changes in the structure and function of brain circuits that can be related to the liability of anxiety disorders[12]. For example, experimental studies show that neuroinflammation induced by lipopolysaccharide injection leads to increased anxiety- and depressive-like behaviors in mice in addition to neurophysiological changes that include the

activation of microglia, secretion of cytokines in the basolateral amygdala, and increased presynaptic glutamate release. Interestingly, pre-treatment with fluoxetine was able to prevent these alterations while alleviating the associated behaviors, suggesting that the resulting behavior outcomes are mediated by enhancing neuroinflammation and glutamatergic synaptic transmission[18].

The relationship between pathways involving axon guidance molecules with the psychiatric outcomes, including the association of the ephrin signaling with GAD, might be explained by the multiple functions of these molecules in both neuron development and in the inflammatory response. Axon guidance is the process during the development of neural circuits by which the neurons navigate to find their target cells and form synapses, with the participation of specific molecules (axon guidance molecules) secreted from glial cells, such as semaphorin, ephrin, and netrin. Besides axon guidance, these molecules have also been implicated in several steps of the neuroinflammatory response, including initial response, glia activation, and resolution of neuroinflammation. Ephrins, for example, act in contact-mediated repulsion during axonomic growth of neural development and in the adult nervous system. They relay bidirectional signals between neurons and glial cells to regulate glial inflammatory activation[15]. Therefore, the suggestive associations with psychiatric traits we reported here might be the result of a mechanism linking neuroinflammation and neurodevelopment through these pathways.

Another reliable association demonstrated by the QQ-plots was between SUD and the GOBP cytokine production involved in immune response gene-set, which shows that the association is being driven by a high proportion of genes comprising this pathway, suggesting that inflammation-related molecular mechanisms might also be related with the susceptibility of SUD as well.

In this context, it would be important to further explore the association between ADHD and axonal guidance-related pathways, considering their relevance during neurodevelopment. Although semaphorin signaling pathways showed nominal associations with ADHD in our study, the pattern observed in the QQ-plots indicates that the signal is likely being driven by a few genes and may not represent the involvement of this pathway as a whole. A similar interpretation can be made for the gene-sets associated with IQ. Although there are interesting signals suggesting that ephrin and semaphorin signaling pathways may be related to cognitive performance, especially in the vocabulary subtest - which measures general intelligence, comprehension, and learning ability - the pathway seems to include many non-contributing genes. It would be important to expand the investigation to identify specific mechanisms or molecules that may be carrying those signals, considering the relevance of these traits in ADHD symptomatology and the shared genetic architecture between the disorder and neurocognitive domains. In addition, this involvement is consistent with the fact that neuroinflammatory processes have been associated with synaptic dysfunction and loss responsible for cognitive decline in neurological diseases[19].

The study should be viewed in light of some limitations. First, the limited sample size may be preventing the identification of important neuroinflammatory pathways for ADHD and related traits. Still, the gene-set approach increases statistical power since it combines the effects of several genes and reduces the number of analyses. Additionally, collecting data on serum levels of inflammatory markers in our sample would allow the analysis of the direct effects of these gene-sets on neuroinflammatory markers in our sample. Since our findings were restricted to a clinical sample of ADHD, the gene-sets associated with the comorbid phenotypes should be tested in populational and/or clinical samples with these phenotypes as primary outcomes. This might clarify if the observed effects are specifically related to the comorbidity or represent a common physiopathological relationship for multiple psychiatric traits.

In conclusion, the present study corroborates previous findings suggesting that neuroinflammation plays an important role in the pathophysiology of psychiatric disorders. Future GWAS should confirm this involvement as larger sample sizes are achieved. Moreover, further exploration of the mechanisms underlying such associations should be pursued to help to elucidate neurobiological aspects of ADHD and related traits.

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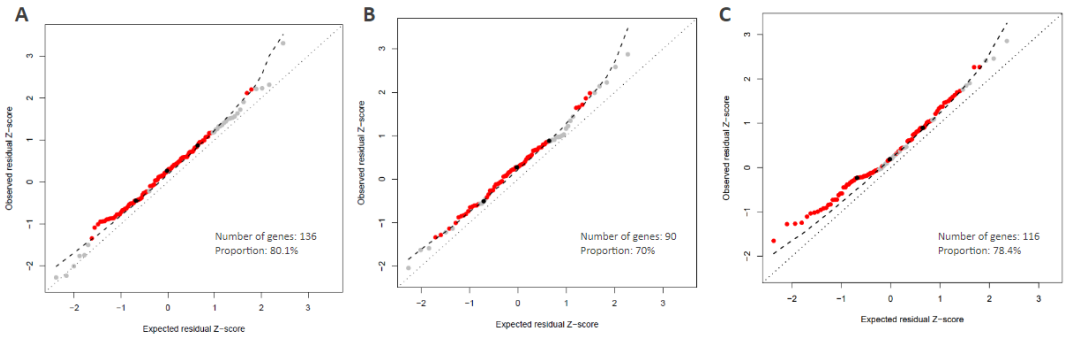


Figure 1. QQ-plots of genes Z-scores from gene-sets associated with Generalized Anxiety Disorder (A and B) and with Substance Use Disorder (C). A) WP_NEUROINFLAMMATION_AND_GLUTAMATERGIC_SIGNALING, B) REACTOME_EPH_EPHRIN_SIGNALING, C) GOBP_CYTOKINE_PRODUCTION_INVOLVED_IN_IMMUNE_RESPONSE. The Y-axis represents the residualised Z-scores from the null model for each gene set, and the X-axis represents the expected values based on the quantiles across all genes in the data. The 25th, 50th and 75th percentiles are indicated by the black points. The dashed black line is a one-sided (upper) 95% confidence band. Red points indicate the genes that surpass the confidence band, while the grey ones represent those below the confidence band. The proportion defines the percentage of genes in the gene-set exceeding the confidence band.

Table 1. Sample characteristics.

	ADHD (n= 407)	**Controls (n=463)**	**P-value**
	n (%)		
Gender (female)	191 (46.9)	241 (52.1)	0.132
Generalized Anxiety Disorder	89 (21.9)	51 (11.1)	<0.001
Major Depressive Disorder	161 (39.6)	132 (28.6)	0.001
Bipolar Disorder	77 (18.9)	14 (3.0)	<0.001
Substance Use Disorder[a]	199 (47.7)	105 (22.7)	<0.001
Oppositional Defiant Disorder	267 (65.9)	18 (3.9)	<0.001
	Mean (SD)		
Age	33.6 (10.8)	29.4 (8.7)	<0.001
Vocabulary subset IQ	105.7 (0.54)	-	-
Block design subset IQ	98.6 (0.79)	-	-

Abbreviations: *ADHD*, attention-deficit/hyperactivity disorder; *SD*, standard deviation; *IQ*, intelligence quotient

[a]Include illicit drugs and nicotine

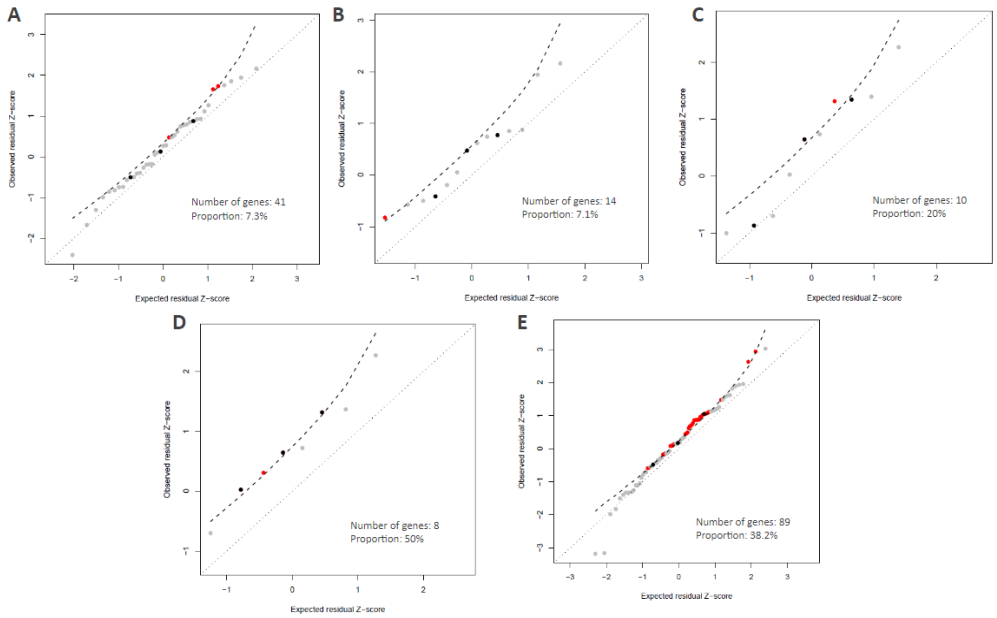
Table 2. Competitive gene-set analysis of neuroinflammation-related pathways and ADHD diagnosis (n = 880) and comorbidities and cognitive performance within the ADHD sample (n = 417).

Gene-sets[a]	**Nº genes**	ADHD[b]	GAD[b]	MDD[b]	BD[b]	SUD[b]	ODD[b]	Vocabulary IQ[b]	Block design IQ[b]
HP neuroinflammation	17	0.9725	0.4473	0.9605	0.2484	0.3271	0.4041	0.1157	0.8630
WP neuroinflammation	11	0.6432	0.7509	0.7910	0.6680	0.6538	0.3523	0.3539	0.0687
WP neuroinflammation and glutamatergic signaling	136	0.5014	**0.0069**	0.2614	0.7813	0.2258	0.8966	0.2106	0.3619
GOBP neuroinflammatory response	66	0.1906	0.8925	0.7627	0.5227	0.4955	**0.0067**	0.4888	**0.0382**
BioCarta cytokine pathway	19	0.4671	0.3198	0.1804	0.7403	0.6180	0.1354	**0.0464**	0.9322
GOBG cytokine production involved in immune response	116	0.1508	0.1014	**0.0210**	0.0810	**0.0304**	0.8960	0.4319	0.8236
GOBP cytokine production involved in inflammatory response	55	0.4915	0.7857	0.3376	0.1952	0.0869	0.9960	0.9361	0.5115
WP cytokines and inflammatory response	25	0.4301	0.4194	0.1299	0.4697	0.6765	0.5487	0.9243	0.8656
GOBP semaphorin plexin signaling pathway	41	**0.0261**	0.7465	0.6552	0.6632	0.5630	0.6307	0.2835	0.4047
GOBP semaphorin plexin signaling pathway involved in neuron projection guidance	14	**0.0058**	0.9442	0.9139	0.7444	0.0631	0.2992	0.6008	**0.0408**
Reactome sema4d in semaphorin signaling	24	0.3248	0.4749	0.2903	0.6205	0.4175	0.7169	**0.0475**	0.8590

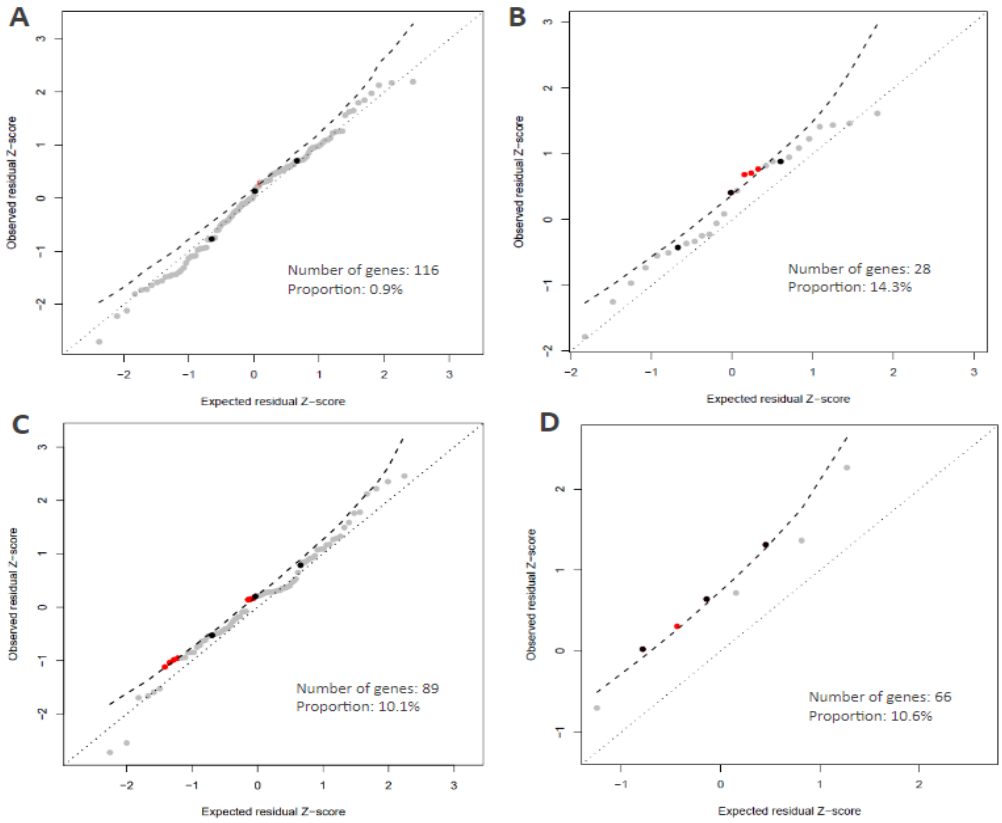
Reactome eph ephrin mediated repulsion of cells	50	0.4926	0.0585	0.5488	0.7274	0.2478	0.1963	**0.0440**	0.0893
Reactome eph ephrin signaling	90	0.4200	**0.0448**	0.7650	0.8932	0.3706	0.1220	**0.0318**	0.1904
Reactome ephrin signaling	17	0.3076	0.5105	0.7853	0.2128	0.8576	0.1455	**0.0060**	0.1232
GOBP netrin activated signaling pathway	10	**0.0468**	0.4722	0.6915	0.3593	0.9746	0.8779	0.4231	0.9170
PID netrin pathway	32	0.2147	0.2965	0.5096	0.3723	0.9132	0.3400	0.1089	0.9391
Reactome netrin 1 signaling	49	0.1157	0.4731	0.8014	0.7795	0.9901	0.3694	0.1637	0.8393
Reactome netrin mediated repulsion signals	8	**0.0123**	0.4955	0.6921	0.6049	0.6110	0.8611	0.6864	0.6060
WP netrinunc5b signaling pathway	50	0.1306	0.1534	0.2215	0.6884	0.2204	0.4008	0.1978	0.6665
Reactome TNF signaling	41	0.3904	0.3841	0.2935	0.3382	0.0642	0.6617	0.9140	0.8456
WP TNF-alpha signaling pathway	89	**0.0461**	0.2992	0.2722	0.5940	**0.0316**	0.1966	0.1713	0.9832
GOBP tumor necrosis factor mediated signaling pathway	99	0.5829	0.2668	0.7389	0.9016	0.2150	0.2732	0.3689	0.8140
ST tumor necrosis factor pathway	28	0.1762	0.5001	**0.0431**	0.1203	0.5708	0.3785	0.4149	0.8135

[a]MSigDB collections searched: 1) Curated gene-sets from Canonical pathways - gene-sets that are usually canonical representations of a biological process designed by domain experts and includes the following pathways databases: Human Phenotype Ontology (HP); WikiPathways (WP); BioCarta, KEGG, PID, Reactome; Signal Transduction knowledge environment (ST); 2) Ontology gene-sets – gene-sets that contain genes annotated by the same ontology term and includes one sub-collection derived from the Gene Ontology resource (GO), which contains Biological Process (GOBP), Cellular Component (GOCC), and Molecular Function (GOMF) components and a second sub-collection derived from the Human Phenotype Ontology (HPO).

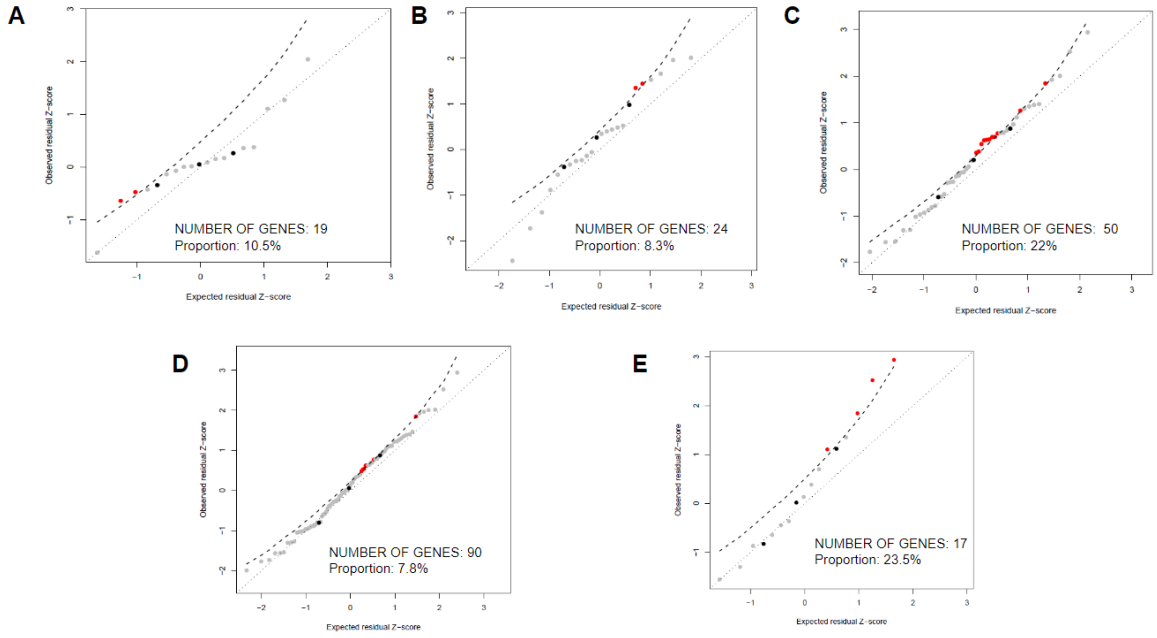
[b]Uncorrected P-values.
Abbreviations: GAD, Generalized Anxiety Disorder; MDD, Major Depressive Disorder; BD, Bipolar Disorder, SUD, Substance Use Disorder; ODD, Oppositional Defiant Disorder



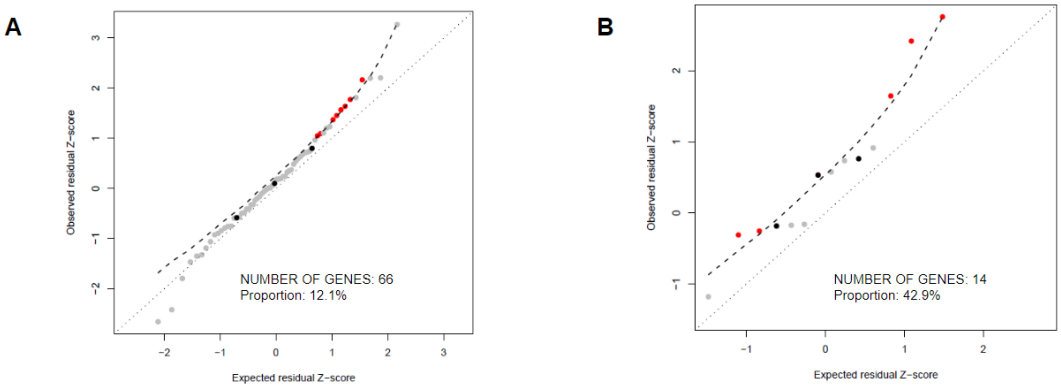
Supplementary Figure 1. QQ-plots of genes Z-scores from gene-sets associated with case-control status. A) GOBP_SEMAPHORIN_PLEXIN_SIGNALING_PATHWAY, B) GOBP_SEMAPHORIN_PLEXIN_SIGNALING_PATHWAY_INVOLVED_IN_NEURON_PROJECTION_GUIDANCE, C) GOBP_NETRIN_ACTIVATED_SIGNALING_PATHWAY, D) REACTOME_NETRIN_MEDIATED_REPULSION_SIGNALS, E) WP_TNFALPHA_SIGNALING_PATHWAY. The Y-axis represents the residualised Z-scores from the null model for each gene set, and the X-axis represents the expected values based on the quantiles across all genes in the data. The 25th, 50th and 75th percentiles are indicated by the black points. The dashed black line is a one-sided (upper) 95% confidence band. Red points indicate the genes that surpass the confidence band, while the grey ones represent those below the confidence band. The proportion defines the percentage of genes in the gene-set exceeding the confidence band.



Supplementary Figure 2. QQ-plots of genes Z-scores from gene-sets associated with Major Depressive Disorder (A and B), Substance Use Disorder (C) and Opposite Defiant Disorder (D). A) GOBP_CYTOKINE_PRODUCTION_INVOLVED_IN_IMMUNE_RESPONSE, B) ST_TUMOR_NECROSIS_FACTOR_PATHWAY, C) WP_TNFALPHA_SIGNALING_PATHWAY, D) GOBP_NEUROINFLAMMATORY_RESPONSE. The Y-axis represents the residualised Z-scores from the null model for each gene set, and the X-axis represents the expected values based on the quantiles across all genes in the data. The 25th, 50th and 75th percentiles are indicated by the black points. The dashed black line is a one-sided (upper) 95% confidence band. Red points indicate the genes that surpass the confidence band, while the grey ones represent those below the confidence band. The proportion defines the percentage of genes in the gene-set exceeding the confidence band.



Supplementary Figure 3. QQ-plots of genes Z-scores from gene-sets associated with QI verbal. A) BIOCARTA_CYTOKINE_PATHWAY, B) REACTOME_SEMA4D_IN_SEMAPHORIN_SIGNALING, C) REACTOME_EPH_EPHRIN_MEDIATED_REPULSION_OF_CELLS, D) REACTOME_EPH_EPHRIN_SIGNALING, E) REACTOME_EPHRIN_SIGNALING. The Y-axis represents the residualised Z-scores from the null model for each gene set, and the X-axis represents the expected values based on the quantiles across all genes in the data. The 25th, 50th and 75th percentiles are indicated by the black points. The dashed black line is a one-sided (upper) 95% confidence band. Red points indicate the genes that surpass the confidence band, while the grey ones represent those below the confidence band. The proportion defines the percentage of genes in the gene-set exceeding the confidence band.



Supplementary Figure 4. QQ-plots of genes Z-scores from gene-sets associated with QI execution. A) GOBP_NEUROINFLAMMATORY_RESPONSE , B) GOBP_SEMAPHORIN_PLEXIN_SIGNALING_PATHWAY_INVOLVED_IN_NEURON_PROJECTION_GUIDANCE. The Y-axis represents the residualised Z-scores from the null model for each gene set, and the X-axis represents the expected values based on the quantiles across all genes in the data. The 25th, 50th and 75th percentiles are indicated by the black points. The dashed black line is a one-sided (upper) 95% confidence band. Red points indicate the genes that surpass the confidence band, while the grey ones represent those below the confidence band. The proportion defines the percentage of genes in the gene-set exceeding the confidence band.

3 CONCLUSÕES E PERSPECTIVAS

De acordo com os resultados apresentados no tópico 2, o presente trabalho corrobora estudos prévios sugerindo que vias neuroinflamatórias e moléculas relacionadas exercem um papel importante na fisiopatologia do TDAH e fenótipos associados.

A partir da interpretação dos gráficos QQ-plots, destacamos as associações que incluem a via de sinalização da efrina e a via de sinalização de neuroinflamação e glutamatérgica com o Transtorno de Ansiedade Generalizada (TAG) e a via de produção de citocinas envolvida na resposta imune e o Transtorno por Uso de Substâncias (TUS) em pacientes com TDAH. Além disso, destacamos que as vias que envolvem moléculas relacionadas a orientação axonal com TDAH, ansiedade e QI podem representar um mecanismo patofisiológico comum entre esses transtornos, apesar de os gráficos das associações indicarem a possibilidade de que estas possam estar sendo levadas por um número menor de genes, e não representar a via como um todo. Em geral, nossos resultados corroboram achados anteriores que sugerem que a neuroinflamação pode causar alterações na estrutura e função dos circuitos cerebrais possivelmente relacionadas ao desenvolvimento desses transtornos e desempenho cognitivo.

Em trabalhos futuros, com o objetivo de compreender melhor as vias de neuroinflamação nos fenótipos relacionados ao TDAH, temos como perspectivas realizar essas análises de gene-sets em uma amostra populacional, para avaliar como se comportam os resultados quando o transtorno avaliado não está em comorbidade com TDAH. Pretendemos explorar de forma mais detalhada e com diferentes abordagens a associação desses transtornos com moléculas de orientação axonal considerando a importância dessas moléculas no neurodesenvolvimento e que nossos resultados apontam para a possibilidade de que genes específicos que participam dessa via podem estar guiando as associações.

Ainda, diferentes abordagens como escores de risco poligênicos (PRS) ou PRS-set, que avaliam como polimorfismos de nucleotídeos únicos de todo o genoma somados e ponderados pelo tamanho de efeito impactam em determinado desfecho, devem ser realizadas para confirmar essa relação entre neuroinflamação e fenótipos psiquiátricos.

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ANEXO - NORMAS DA REVISTA - BRAZILIAN JOURNAL OF PSYCHIATRY

Manuscript preparation

Manuscripts are accepted for consideration by the *Brazilian Journal of Psychiatry* based on the understanding that they are original, are not being considered for publication elsewhere, and have not been published previously. The final version of the submitted manuscript should have been approved by all authors.

Manuscript types and word limits

The table below shows the types of manuscript accepted for evaluation and the maximum number of words (from Introduction to end of Discussion), references and tables/figures allowed for each category.

Manuscript type	Main text words	Abstract words	References	Tables+boxes+figures
Original Articles	5000	Structured, 200	40	6
Review Articles	6000	Structured, 200	Unlimited	6
Brief Communications	1500	Structured, 200	15	2
Special Articles	6000	Unstructured, 200	Unlimited	6
Letters to the Editors	500	No abstract	5	1
Editorials	900	No abstract	5	1

- **Original articles:** These should describe fully, but as concisely as possible, the results of original research, containing all the relevant information for those who wish

to reproduce the research or assess the results and conclusions. Original articles should have the following sections: Introduction, Methods, Results, and Discussion. The last paragraph(s) of the Discussion section should address study limitations and concluding remarks, but without separate subtitles.

- **Review articles:** These should be systematic reviews and should include critical assessments of literature and data sources, critically reviewing and evaluating existing knowledge on a designated topic, in addition to commenting on studies by other authors. The search strategy and selection process should be described in detail, according to PRISMA or other appropriate guidelines. The main text may follow a structure similar to that of an original article, or may be adapted to better reflect the presentation of findings. Non-systematic reviews should be submitted in the Special Articles category.
- **Brief communications:** Original but shorter manuscripts addressing topics of interest in the field of psychiatry, with preliminary results or results of immediate relevance. The main text should use the same subtitles described for original articles above.
- **Special articles:** Articles that address specific current topics relevant to clinical practice and are less comprehensive than review articles. These include non-systematic reviews and critical assessments of the literature, reviewing and evaluating existing knowledge on a designated topic. In this category, authors are free to decide upon the article's structure and to use the subtitles that better reflect the contents of their contribution.
- **Letters to the Editors:** Letters can contain reports of unusual cases, comments on relevant scientific topics, critiques of editorial policy, or opinions on the contents of the journal (maximum of four authors).
- **Editorials:** Critical and in-depth commentary invited by the editors or written by a person with known expertise in the topic.

Title page

Page 1 should contain a full title (max. 150 characters, specific, informative, attractive, no abbreviations), authors' names in the form that is wished for publication, their departments and institutions, including city and country. Please also include a running title with a maximum of 50 characters (letters and spaces) and inform of any previous presentations of the manuscript, if applicable (e.g., in abstract or preprint form). The full name, telephone number, e-mail address and full postal address of the corresponding author should be stated.

Abstract

Page 2 should present a structured abstract (where applicable; check table above with abstract requirements for each manuscript type), in English only, with the following sections: Objective, Methods, Results, and Conclusions. Please indicate three to five keywords in strict accordance with MeSH, and avoid repeating words from the title. If submitting a randomized clinical trial, inform the clinical trial registration number at the end of the abstract (see below).

Clinical Trial Registration: The *Brazilian Journal of Psychiatry* will only accept clinical trials that have been registered in a public registry that meets the World Health Organization (WHO) and ICMJE requirements.

Main text

The manuscript file (Main Document) must be written in English, double-spaced throughout, and should contain the following sections in this order: title page, abstract, manuscript text, acknowledgments (individuals, non-commercial funding agencies, etc.), disclosure (potential conflicts of interest covering the last 3 years, commercial funding sources), references, figure legends, and tables. Use 10-, 11-, or 12-point font size. Abbreviations should be avoided and limited to those considered "standard." All abbreviations should be spelled out at first mention in the text and also in table/figure legends. All units should be metric. Avoid Roman numerals. Generic names of drugs should be used.

The Methods section must include information on ethics committee approval. Studies involving humans must provide details about informed consent procedures, and studies involving animals must describe compliance with institutional and national standards for the care and use of laboratory animals. Patient anonymity should be guaranteed.

References

Authors are responsible for the accuracy and completeness of their references and for correct in-text citation. An EndNote style file can be downloaded here. Number references consecutively in the order they appear in the text using superscript Arabic numerals; do not alphabetize. References cited only in tables or figure legends should be numbered in accordance with the first citation of the tables/figures in the text, i.e., as though they were part of the text.

Please observe the style of the examples below. To include manuscripts accepted, but not published, inform the abbreviated title of the journal followed by "Forthcoming" and the expected year of publication. Journal titles should be abbreviated in accordance with Index Medicus. Personal communications, unpublished manuscripts, manuscripts submitted but not yet accepted, and similar unpublished items should not be cited; if absolutely essential, bibliographic details should be described in the text in parentheses.

Examples:

- **Journal article:** Coelho FM, Pinheiro RT, Silva RA, Quevedo LA, Souza LD, Castelli RD, et al. Major depressive disorder during teenage pregnancy: socio-demographic, obstetric and psychosocial correlates. Braz J Psychiatry. 2013;35:51-6. List all authors when six or fewer. When there are seven or more, list only the first six authors and add "et al."
- **Book:** Gabbard GO. Gabbard's treatment of psychiatric disorders. 4th ed. Arlington: American Psychiatric Publishing; 2007.
- **Book chapter:** Kennedy SH, Rizvi SJ, Giacobbe P. The nature and treatment of therapy-resistant depression. In: Cryan JF, Leonard BE, editors. Depression: from psychopathology to pharmacotherapy. Basel: Karger; 2010. p. 243-53.
- **Theses and dissertations:** Trigeiro A. Central nervous system corticotropin releasing factor (CRF) systems contribute to increased anxiety-like behavior during opioid withdrawal: an analysis of neuroanatomical substrates [dissertation]. San Diego: University of California; 2011.
- **Electronic articles and web pages:** World Health Organization. Depression and other common mental disorders: global health estimates [Internet]. 2017 [cited 2020 May 11]. https://www.who.int/mental_health/management/depression/prevalence_global_health_estimates/en/

Illustrations (figures, tables, boxes)

Illustrations (figures, tables, or boxes) should clarify/complement rather than repeat the text; their number should be kept to a minimum. All illustrations should be submitted on separate pages at the end of the manuscript, following the order in which they appear in the text and numbered consecutively using Arabic numerals. Descriptive legends should be included for each illustration in the main text file, and any abbreviations or symbols used should be

explained using these footnotes: ┼ ╬ § ‖ ¶ ┼┼ ╬╬ etc. Asterisks should be reserved for the expression of significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Illustrations extracted from previously published works should be accompanied by written permission for reproduction from the current copyright holder at the time of submission.

Tables and boxes should preferably be submitted in Word format, appended to the end of the manuscript text file (after any figure legends), rather than uploaded as separate files. However, Excel files are also accepted. If using Excel, do not place tables on individual spreadsheets within the same file because only the first sheet will be visible in the converted PDF. In tables, each cell should contain only one item of data; subcategories should be in separate rows and cells (i.e., do not use Enter or spaces inside a cell). Tables containing data that could be given succinctly in 1-2 sentences should be converted to text. Large or detailed tables may be submitted separately as online-only supplementary material (see details below).

Figures should be submitted in one of the following acceptable file formats: AI, BMP, DOC, EMF, EPS, JPG, PDF, PPT, PSD, TIF, WMF, and XLS. Figures can be included in the manuscript, but preferably should be uploaded as separate files. If your manuscript is accepted, you may be asked to provide high-resolution, uncompressed TIF files for images, as well as open/editable versions of figures containing text, to facilitate copyediting (e.g., flowcharts made in Word or PowerPoint). Supporting figures may be submitted separately as online-only supplementary material.

Online-only supplementary material

Supporting materials (text, tables, figures) for online-only publication should be submitted as a single Word document with pages numbered consecutively. Each element included in the online-only material should be cited in the main text and numbered in order of citation (e.g., Supplementary Methods, Table S1, Table S2, Figure S1, Figure S2, etc.). The first page of the online-only document should list the number and title of each element included in the document. The editors may select material submitted for publication in the print version to be posted online only.